nature ecology & evolution

SUPPLEMENTARY INFORMATION
https://doi.org/10.1038/s41559-020-1122-9

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A one-billion-year-old multicellular chlorophyte

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Supplementary Information for

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This file includes:

Supplementary Table

Supplementary Table 1 | Morphological comparison between *Proterocladus* and extant bacterial and eukaryotic analogs. "√" denotes presence, "X" denotes absence.

	Fossil	**Chlorophyta**			**Cyanobacteria**	**Fungi**	**Xanthophyta**	**Rhodophyta**
Characters	*Proterocladus*	*Rhizoclonium*	*Cladophora*	*Cladophoropsis*	*Nostochopsis*	*Neurospora* hyphae	*Vaucheria*	*Griffithsia*
Uniseriate filament	√	√	√	√	√	√	√	√
Upward thallus growth	√	√	√	√	√	√	√	√
True lateral branch	√	√	√	√	√	√	√	√
Apical cell division	√	√	√	√	√	√	√	√
Erect epibenthic habit	√	√	√	√	√	√	√	√
Tuft formation/aggregation	√	√	√	√	√	√	√	√
Constriction at septum	√	√	√	√	√	√	X	√
Centripetal invagination	√	√	√	√	√	√	√	√

Akinete	√	√	√	X	√	X	√	√
Lateral pore	√	√	√	√	√	X	√	X
Cell width variation along filament length	√	√	√	√	√	X	√	√
Intercalary cell division	√	√	√	√	√	X	√	√
Holdfast	√	√	√	√	X	√	√	√
Siphonocladous construction	√	√	√	√	X	√	√	√
Sexual reproduction	√	√	√	√	X	√	√	√
Cell length variation	√	√	√	√	X	X	X	√
Inflated apical cell	√	√	X	X	X	X	X	√
Narrow apical extension	√	X	X	X	X	X	√	X
Segregative cell division	X	X	X	√	X	X	X	X
Tenacular cell	X	X	X	√	X	X	X	X
Outer sheath	X	X	X	X	√	X	X	X

Septal pore	X	X	X	X	X	√	√	√
Loop structure/hyphal fusion	X	X	X	X	X	√	X	X
Multiple branches at one cell	X	X	X	X	X	√	X	√
Reference	1	2,3	4	5	6	7,8	9,10	11

Stratigraphy and sedimentary environment

Late Mesoproterozoic to Neoproterozoic sedimentary rocks are widely exposed in southern Liaoning Province, North China (Extended Data Fig. 1). These rocks represent fluvial to shallow marine deposits with a total thickness of up to 12.7 km[12]. Regionally, the sedimentary sequence sits unconformably on the metamorphosed Archean and Paleoproerozoic basement, and is disconformably overlain by the Dalinzi Formation and the lower Cambrian Jianchang Formation[13]. Lithologically, the sequence is divided into, in ascending order, the Yongning Group, Xihe Group, Wuhangshan Group, and Jinxian Group[14]. The Yongning Group, 466.7–6,000 meters in thickness, is characterized by purple and grey thick-bedded quartz sandstone with conglomerate interbeds and well-developed cross bedding structures, representing fluvial and alluvial deposits[15]. The conformably overlying Xihe Group is divided into the Diaoyutai, Nanfen, and Qiaotou formations, in ascending order. The Diaoyutai Formation, 57.2–260 meters in thickness, is mainly composed of light gray glauconitic quartz sandstone with ca. 1-m-thick conglomerate at base. Cross bedding structures are well-developed, suggesting dominate littoral depositional environment[16]. The Nanfen Formation, from which the *Proterocladus* fossils reported here were collected, is 254–560-m-thick and conformably overlies the Diaoyutai Formation. It mainly consists of yellowish green silty shale and mudstone in the lower part; greyish thick-bedded argillaceous limestone in the middle; and greyish green shale with sandstone interbeds in the upper part. The tripartite Nanfen sediments indicate oscillation in depositional environment between subtidal and carbonate platform facies. The conformably overlying Qiaotou Formation, 174–1,527 meters in thickness, is mainly composed of thick- to thin-bedded quartz sandstone with well-developed cross-beds, representing intertidal deposits. The overlying Wuhangshan Group consists of three units, the Changlingzi, Nanguanling, and Ganjingzi formations, in ascending order. The Changlingzi Formation, which conformably sits on the Qiaotou Formation, is 317–1,537 meters in thickness. It is characterized by yellowish green shale in the lower part; yellowish green sandstone with shale and siltstone interbeds in the middle; and glauconitic sandstone and argillaceous limestone interbeds in the upper part. Horizontal bedding is common in the lower part, but cross bedding becomes abundant in the upper part, suggesting shallow marine deposits. The Nanguanling Formation is in conformable contact with the underlying Changlingzi Formation. The Nanguanling Formation, 147–797-m-thick, is mainly composed of dark grey limestone with stromatolites in the upper part, probably deposited in an inner carbonate platform facies. The Ganjingzi Formation, 409–906-m-thick, is in conformable contact with the underlying Nanguanling Formation. It mainly consists of light grey thick-bedded dolostone with stromatolites, likely representing deposition in a restricted platform facies[17]. The conformably overlying Jinxian Group is mainly composed of carbonate in the lower and middle parts with a gradual transition to siliciclastics in the upper part, indicating an environmental transition from carbonate platform facies to shallow marine littoral facies[18]. Briefly, the Jinxian Group consists of Yingchengzi, Shishanlitai, Majiatun, Cuijiatun, Xingmincun, and Getun formations, in ascending order. The Yingchengzi Formation, 117–894-m-thick, is mainly composed of dark grey thick-bedded micritic and stromatolitic limestone in the lower part and crystalline limestone in the upper part. The conformably overlying Shisanlitai Formation, 57–278-m-thick, consists of dark grey medium-bedded micritic limestone and stromatolitic limestone in the lower part and yellowish green shale with stromatolitic limestone interbeds. Reddish stromatolites are common. The Majiatun Formation, which conformably sits on the Shisanlitai Formation, is 110–178 meters in thickness. It is mainly composed of thick-bedded stromatolitic limestone in the lower part and micritic limestone in the upper part. In contrast, the conformably overlying Cuijiatun Formation, 20–148-m-thick, is characterized by greyish green silty shale and mudstone. Stromatolitic limestone develops in places in the upper part. Similarly, the conformably overlying Xingmincun Formation is 121–341 m thick and composed of dark grey silty shale and mudstone in the lower part; yellowish green and purple shale in the middle; and dark grey micritic limestone in the upper part. The Getun Formation disconformably sits on the Xingmincun Formation and has a thickness of 38–83 meters. It is mainly composed of dark grey shale with quartz sandstone interbeds. The Dalinzi Formation, which disconformably overlies the Getun Formation and disconformably underlies the early Cambrian Jianchang Formation, is 63–121-m-thick and mainly composed of greyish and purple quartz sandstone in the lower part and light grey dolostone and limestone in the upper part with halite pseudomorphs, indicating evaporitic tidal flat and coastal Sabkha facies[19].

Age constraints

Abundant carbonaceous compression macrofossils have been reported from the Xihe, Wuhangshan, and Jinxian groups. Taken together, these fossils suggest a depositional age of late Mesoproterozoic to early Neoproterozoic (or Tonian). The *Chuaria-Tawuia* assemblage occurs abundantly in the Diaoyutai, Nanfen, Changlingzi, Nanguanling, and Getun formations[14] (Extended Data Fig. 1). In addition, many tubular fossils with transverse annulations, including *Protoarenicola* and *Pararenicola*, have been reported from the Changlingzi, Nanguanling, and Getun formations[20] (Extended Data Fig. 1). Although the *Chuaria-Tawuia* assemblage has been known throughout the Proterozoic Eon, it is most common in early Neoproterozoic or Tonian deposits[21]. More importantly, *Protoarenicola* and *Pararenicola* have only been reported from the early Neoproterozoic so far[22,23], suggesting that the Wuhangshan and Jinxian groups are likely Tonian deposits. The underlying Xihe Group may also be early Neoproterozoic or slightly older given that no significant depositional hiatus is observed. Thus, biostratigraphic data broadly constrain the Xihe, Wuhangshan, and Jinxian groups to late Mesoproterozoic–early Neoproterozoic in age. In contrast, the depositional age of the Dalinzi Formation is rather ambiguous and controversial for the lack of diagnostic fossils or reliable radiometric ages.

Consistent with the biostratigraphic data, available geochronological data also indicate a late Meoproterozoic–early Neoproterozoic age for the sequence from the Yongning Group to the Jinxian Group in southern Liaoning Province. A number of whole-rock K-Ar and Rb-Sr ages have been published for the Yongning–Jinxian groups[24], but these ages have large uncertainties and are unreliable because of the unpredictable behaviors of the K-Ar and Rb-Sr systems[25]. Recently, three independent studies revealed youngest detrital zircon ages of 1,056 ± 22 Ma (LA-ICP-MS)[25], 1,075 ± 27 Ma (SHRIMP)[26], and 1,136 ± 11 Ma (LA-ICP-MS)[27] from the Diaoyutai Formation, placing maximum age constraints on the Xihe Group and the Nanfen Formation that contains the *Proterocladus* fossils reported in this paper. Multiple diabase sills emplaced in different units of the Xihe, Wuhangshan, and Jinxian groups have been recently dated using zircon LA-ICP-MA U-Pb and baddeleyite SIMS Pb-Pb techniques. These sills give early Neoproterozoic ages, for example, 923 ± 22 Ma (zircon) and 909 ± 7 Ma (baddeleyite) from the Qiaotou Formation[12], 947.8 ± 7.4 Ma (zircon) from the Qiaotou Formation[27], 924 ± 28 Ma (zircon) and 924 ± 5 Ma (baddeleyite) from the Cuijiatun Formation[12], and 900 ± 34 Ma (zircon) and 886 ± 5 Ma (baddeleyite) from the Xingmincun Formation. These dates provide minimum constraints on the depositional age for the Nanfen Formation. Therefore, the Nanfen Formation can be constrained between 1,056 Ma and 947.8 Ma (Extended Data Fig. 1). Based on a compilation of radiometric dates from Neoproterozoic successions in North China, Zhao et al. conclude that the Nanfen is between ca. 1120 Ma and ca. 945 Ma[27]. Given that the total thickness of the Xihe-Wuhangshan-Jinxian sequence is up to 12.7 km, and *Proterocladus* occurs in the basal to lower part of the Nanfen

Formation that conformably overlies the Diaoyutai Formation, the first occurrence of *Proterocladus* in the Nanfen Formation is probably ca. 1,000 Ma.

Proterocladus has been previously reported from the Tonian Svanbergfjellet Formation in Svalbard[1] and Khastakh Formation in Siberia[28]. The Svanbergfjellet Formation is not directly constrained by radiometric ages. However, a negative $\delta^{13}C_{carbonate}$ excursion, which begins in the underlying Upper Member of the Grusdievbreen Formation and ends in the lower Dolomite Member of the Svanbergfjellet Formation[29-31], has been correlated with the Bitter Spring Excursion that is constrained between ca. 811.5 and 789 Ma[29,32,33]. Given that all Svalbard specimens of *Proterocladus* were recovered from the Upper Algal Dolomite Member of the Svanbergfjellet Formation above the end of the Bitter Spring Excursion[1], the Svanbergfjellet occurrence of *Proterocladus* should be younger than 811.5 Ma and likely even younger than 789 Ma. In addition, abundant organic-walled microfossils of *Cerebrosphaera globosa* (=*Cerebrosphaera buickii*) have been recovered from the Algal Dolomite Member of the Svanbergfjellet Formation and co-occur with *Proterocladus*[1]. *Cerebrosphaera globosa* has been proposed as a late Tonian index fossil whose global occurrences are constrained between ca. 800 Ma and 729 Ma and probably between ca. 792 and 738 Ma[34]. Thus, in accordance with the chemostratigraphic implication, the occurrence of *C. globosa* also indicates that *Proterocladus* from the Svanbergfjellet Formation is clearly younger than 800 Ma.

Similarly, the depositional age of the Khastakh Formation in Siberia is not directly constrained by radiometric ages, either. However, the organic-walled microfossil *Cerebrosphaera globosa* have been abundantly recovered from the Khastakh Formation and co-occur with *Proterocladus*[28], suggesting that the occurrence of *Proterocladus* from the upper Khastakh Formation is also younger than 800 Ma. Therefore, the Nanfen specimens represent the oldest occurrence of *Proterocladus*, ca. 200 Myr older than previously reported occurrences of this genus.

Taphonomy

Specimens of *Proterocladus* are preserved as carbonaceous compressions in shales and mudstones of the Nanfen Formation (Extended Data Figs. 2–7). They often occur abundantly on bedding surfaces (Extended Data Fig. 8). Energy dispersive X-ray spectroscopy (EDS) point analysis of a *Proterocladus* specimen shows prominent peaks of C, O, Al, and Si (Extended Data Fig. 9a–c). EDS elemental maps show that there is a distinct enrichment in C and deficiency in O, Al, and Si in the fossil relative to the surrounding matrix (Extended Data Fig. 9d), thus indicating that the preserved fossil is mainly composed of organic carbon. This is consistent with the back-scattered electron SEM observation that the fossil appears darker than the matrix (Extended Data Fig. 9b).

Proterocladus specimens in the Nanfen Formation are typically concentrated in thin layers of shales or mudstones. The fossiliferous layers (hereafter referred as fossil layers) are darker in color than the non-fossil layers (hereafter referred as background layers) (Extended Data Fig. 9e–g). The fossil layers possibly represent event beds (e.g., distal turbidites) that rapidly buried fossil remains. *Proterocladus* is often the dominant taxon in fossil layers, and specimens are randomly distributed in fossil layers (Extended Data Fig. 8). The density of fossils is also seen in a polished slab cut perpendicular to the bedding surface (Extended Data Fig. 9h, i). Most *Proterocladus* specimens are preserved as fragments, with only a few preserved intactly (Extended Data Figs. 2–3), suggesting that post-mortem transport over a short distance may have occurred before algal remains were buried. This is also consistent with the fact that the holdfast of *Proterocladus* is rarely preserved (Fig. 1l; Extended Data Fig. 4) and the possibility that the fossil layers may represent microturbidites. To summarize, *Proterocladus* may have lived in a coastal marine environment and its preservation was facilitated by event deposition that transported and quickly buried algal remains entrained in the event deposits.

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